

Haugland and Yue
Serial No. 09/557,275

REMARKS

The Claimed Invention

The claimed invention is directed to unsymmetrical cyanine dyes incorporating an aza-benzazolium moiety. These compounds form fluorescent complexes with nucleic acids and poly(amino acids) and are used to effectively stain such structures.

The Pending Claims

Prior to entry of the attached amendments, Claims 1-47 are pending. Claims 1-14 are directed to unsymmetrical cyanine dyes of the invention. Claims 15-23 are directed to a fluorescent complex comprising a nucleic acid polymer and the cyanine dyes of Claims 1-14. Claims 24-27 are directed to complex comprising a poly(amino acid) and cyanine dyes of Claims 1-14. Claims 28-38 are directed to methods for staining poly(amino acids). Claims 39-43 are directed to methods for staining nucleic acids. Claims 44-47 are directed to kits comprising cyanine dye compounds of Claims 1-14.

The Office Action

Claims 1-47 are restricted.

Amendments

Claims 1-16, 18-24, 26-27, 44 and 46-47 have been amended.

Claims 1, 15, 24, 28, 39 and 44 have been amended to correct a typographical error in the structure as drawn. Support can be found in original Claim 2 as filed and on 6 line 27; p. 57 lines 26-31 to p. 58 lines 1-24; p. 66 compound 14 and 15; p. 67 compound 16 and 17; p. 68 compound 18-20; p. 69 compound 21, 44 and 22; p. 70 compound 23; p. 71 compound 24 and 25; p. 72 compound 26 and 27; p. 73 compound 47 and 28; p. 74 compound 45 and 46; p. 75 compound 29 and 30; p. 76 compound 41 and 31; p. 77 compound 32 and 33; p. 78 compounds 34-37; p. 79 compounds 38-40 and 42; p. 80 compound 43 and 48; p. 81 compound 49 and 50; p.

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82 compound 51 and 52; p. 83 compound 83 and 54; p. 84 compound 55; p. 85 compound 56 and 57; p.86 compound 58 and 59; p. 87 compound 60.

Claims 2-14, 16, 18-22, 25-27, and 46-47 have been amended to replace 'A' with 'The' to indicate dependent form.

Claims 1-3, 5-6, 11-12, 14-15, 22, 24, 44 and 47 have been amended to clarify the Markush groups.

Claims 1, 15, 19, 24 and 44 have been amended to accurately define the A moiety of the claimed compounds. Support can be found in original filed claim 2 and page 4 lines 14-20.

Claims 1, 15, 19, 24 and 44 have been amended include the substituents hydrogen and methylthio of the aromatic carbon atoms of the A moiety. Support can be found page 5 lines 16-22; page 65 compound 11 and page 78 compound 35.

Claims 1, 15, 19, 24 and 44 have been amended to include the definition of β . Support can be found on page 5 lines 5-14.

Claims 1, 15, 19, 24 and 44 have been amended to replace the R2 substituent 'sulfonate' with 'sulfo'. Support can be found on page 5 line 26.

Claims 1, 15, 19, 24 and 44 have been amended to replace $-(CH_2)_2-V-(CH_2)_2-$ with $-(CH_2)_2-W-(CH_2)_2-$. Support can be found on page 8 line 24.

Claims 1, 15, 19, 24 and 44 have been amended to accurately define the ring structure that can be formed by R^6 and R^7 . Support can be found on page 9 lines 14-26; page 10 lines 1-10 and original filed claim 2.

Claims 1, 7-9, 11, 14-15, 19, 24 and 44 have been amended to replace BRIDGE-DYE with BRIDGE. The scope of the claims has now been limited to monomers of a dye or dimmers comprising DYE. Support can be found in the original filed claims and on page 18 lines 20-23; page 19 lines 1-7 and lines 30-31; page 20 lines 1-2.

Claim 3 has been amended to accurately define the substituents of alkyl substituted R^{21} , R^{22} or R^{23} . Support can be found on page 13 lines 30-31; page 14 lines 1-4.

Claim 5 has been amended to accurately define CYCLIC SUBSTITUENT. Support can be found on page 11 lines 31; page 12 lines 1-7.

Claim 6 has been amended to accurately define TAIL. Support can be found in original filed claim 3 and page 12 lines 11-22 and page 13 lines 20-22.

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Claim 12 has been amended to include 'a DNA-binding protein'. Support can be found in original filed claim 13 and page 24 line 30.

Claim 15 has been amended to clarify that that fluorescent complex comprises a) a compound and b) a nucleic acid polymer. Support can be found in the original filed claim and page 3 lines 20-22; page 39 lines 7-24; page 87 Example 32; page 89 Example 33; page 90 Example 34; page 91 Example 35; page 92 Example 36; and page 94 Example 37.

Claim 16 has been amended to include the imitation of nucleic acid polymer fragments. Support can be found on page 32 lines 8-12.

Claim 17 is cancelled without prejudice.

Claim 18 has been amended to clarify the samples comprising nucleic acid polymers. Support can be found on page 32 lines 23-26 and page 39 lines 30-31.

Claim 19 has been amended to clarify the medium that the fluorescent nucleic acid complex can be present in. Support can be found on page 39 lines 21-23.

Claim 21 has been amended to include a cell as a biological structure. Support can be found in original filed claim 20 and page 32 lines 23-28.

Claim 23 is cancelled without prejudice.

Claim 24 has been amended to clarify that the claimed composition is a complex of the aza cyanine dye and a poly (amino acid). Support can be found in the original filed claim and page 3 lines 22-23; page 42 lines 4-6; page 94 Example 38; page 95 Example 39 and Example 40.

Claims 28-43 have been provisionally withdrawn.

Claim 44 has been amended to clarify that the claimed kit contains a stock solution and a buffer for dilution of the stock solution and that the stock solution comprises one or more compounds. Support can be found in original filed Claim 44 and 45; page 51 lines 21-30 and page 52 lines 4-6.

Claim 45 is cancelled without prejudice.

Support for new Claims 48-74 is found in the claims as filed and throughout the specification, and in particular the limitations associated with the compounds of claims 52-74 can be found in compound 14 (page 66); compound 15 (page 66); compound 16 (page 67);

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compound 44 (page 69); compound 25 (page 71); compound 27 (page 72); compound 47 (page 73); compound 28 (page 73); compound 46 (page 74); compound 35 (page 78); and compound 40 (page 79). Additional support for the staining solution of Claims 69-74 can be found on pages 29-31. Support for new claim 48 can be found in original filed claim 44 and page 19 lines 30-31. Support for new claims 49 and 50 can be found on page 51 lines 21-25.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

RESPONSE TO THE RESTRICTION REQUIREMENT

In the response that follows, the Examiner's Election/Restriction of the Applicant's claimed invention is provided in full text, as identified by indented small bold print, followed by the Applicants response.

35 U.S.C. 121 Restriction

- I. **Claims 1-27 and 44-47 drawn to azo dye compositions, classified in class 532, subclass 573 for example.**
- II. **Claims 28-43, drawn to a method of staining nucleic acids, classified in class 436, subclass 94 for example.**

Applicants respectfully traverse the above restriction requirement and request reconsideration. As required by CFR 1.143, Applicants provisionally elect Group I as drawn to the azo dye compounds.

The invention of group I, and the invention of group II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product claimed can be used in a materially different process of using the product (MPEP § 806.05(h)). In the instant case nucleic acids may be tagged or labeled with fluorescent dyes other than azo cyanine dyes, for example, they may be labeled with rhodamine or fluorescein. Further nucleic acids may also be tagged or labeled with oligonucleotide fluorescent intercalating agents or with lanthanide elements for detection with or without the invention of the azo cyanine dyes of group I.

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Applicants respectfully submit while additional compounds other than compounds of the present invention may be used to 'tag or label' nucleic acids, the compounds of the present invention possess unique properties that exclude the use of the Examiner's cited compounds for such methods. Among these unique properties include: (1) increased fluorescence upon formation of a complex, (2) cell permeability properties, (3) wavelength emission spectra and (4) formation of dimmers that permits the staining of an analyte of interest such as nucleic acid polymers in the instant methods.

First, the compounds of the present invention are virtually non-fluorescent when in solution and become fluorescent only after forming a complex with an analyte of interest. When used in methods to stain an analyte of interest in a blot or gel, this unique property overcomes the need for a washing step, providing a method that requires less time than traditional stains that require stringent washing steps to remove unbound fluorescent compounds. In addition, this increased fluorescence property allows for staining of nucleic acids in solution or in another medium wherein the nucleic acid is not attached to a solid or semi-solid surface. This is an application not possible with the compounds cited by the Examiner.

Second, the compounds of the present invention are cell-permeant, especially when present as monomers. Such cell permeability, in conjunction with the ability to increase fluorescence when present as a complex, permits the compounds of the present invention to be used in live cells. Staining an analyte of interest in a live cell offers many applications for the compounds of the present invention such as functioning as tracers or viability markers. The properties of the instant compounds also importantly allows for these compounds to be used in functional assays where removal of unbound compounds is not feasible. The compounds cited by the Examiner cannot be used for such methods.

Third, the compounds of the present invention possess unique wavelength emission spectra not observed by the compounds cited by the Examiner. This unique property is facilitated by the aza-benzazolum moiety and permits the compounds of the present invention to be used in methods that would not be possible with the compounds cited by the Examiner. For example, the compounds of the present invention can be combined with dyes of different wavelengths for use in multiple dye staining techniques whereby the emission from different dyes can easily be distinguished from each other. As the compounds of the present invention

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possess unique wavelength spectra, they effectively increase the multiplexing applications that can be performed. The compounds cited by the Examiner have different wavelengths than the compounds of the present invention and are not typically used in multiplexing applications.

Fourth, the compounds of the present invention can be used in the instant methods when present as dimers. The dimers possess a unique property of a more stable complex with an analyte of interest than the monomers of the present invention. This is an important advantage because it allows for preformed complexes of the compounds and an analyte of interest. This unique property serves two purposes: (1) it allows for a quick determination of the relative amount of analyte of interest and (2) it allows further analysis due to the stable complex formation wherein the complex could be analyzed by gel electrophoresis. For example, a sample thought to contain an analyte of interest could be mixed with a dimer of the present invention that would (1) indicate whether the analyte of interest was present due to the enhanced fluorescence when a complex is formed and if present in a satisfactory amount (2) and allow further analysis on a gel. Thus, the dimers used, in combination with the unique property of the compounds of the present invention of being virtually non-fluorescent when in solution allows for methods not possible with the compounds cited by the Examiner.

Thus, the compounds of the present invention possess many unique properties that are not present in the compounds cited by the Examiner. Moreover, the compounds cited by the Examiner are not feasible compounds for the present methods. Therefore, due to the unique properties of the present compounds, as described above, Applicants respectfully request that the Examiner reconsider the restriction requirement and rejoin the claims of Group I with Group II.

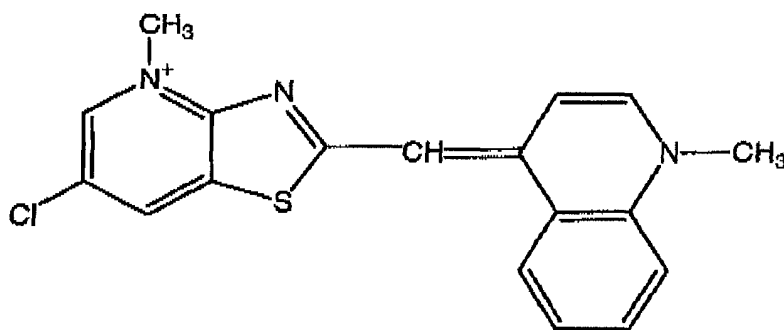
This application contains claims directed to the following patentably distinct species of the claimed invention: α , β , A, ψ , L, R₂-R₃₂, Rx, Sc, V, X, Y, Ym, Yp, m, n, p, CAP, CAP', TAIL, BRIDGE, BRIDGE-DYE, CYCLIC SUBSTITUENT, SPACER, SPACER' SPACER-CAP, AND LINK-SPACER-CAP.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1-47 are generic.

Applicant is advised that a reply to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

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Applicants provisionally elect the following compound 4-(6-chloro-2,3-dihydro-4-methyl(thiazolo[4,5-b]pyridin-2-yl)methylidene)-1-methylquinolinium tosylate. This compound is exemplified as Compound 40 on page 79 of the specification and is covered in Claims 1, 2, 4, 10, 52-54 and 58 of the above-identified application. The elected species compound has the following structure:



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CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 984-5656.

Respectfully submitted,

Date: October 16, 2002

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PATENT**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Haugland and Yue)

Serial No.: 09/557,275)

Filed: April 24, 2000)

For: Aza-Benzazolum Containing
Cyanine Dyes)

Examiner: C. Maupin)

Group Art Unit: 1637)

**SUPPLEMENTAL MARKED-UP
VERSION OF THE CLAIMS**Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

Dear Sir:

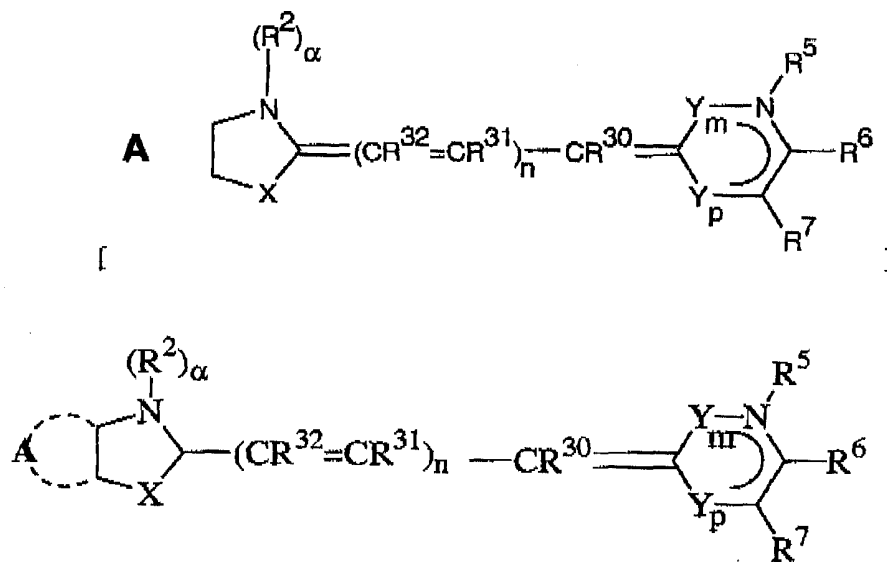
The following Supplemental Marked-up Version of the Claims is hereby submitted together with a Supplemental Clean Version of the Claims and the Supplemental Response to Restriction Requirement.

CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HEREWITH ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADEMARK OFFICE ON 10/16/02 TO
1.703.746.5147 By Julie Eskandari

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1. (Amended) A compound [of the] having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R²)^β, provided at least one of [which] said ring atoms is [a nitrogen atom] N(R²)^β, said ring or rings being] wherein aromatic carbon atoms are optionally [further] substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, [or] -L- -R_x]; or] and -L-S_c;

X is selected from the group consisting of O, S, Se, NR¹⁵, [or] and CR¹⁶R¹⁷, wherein R¹⁵ is [H] hydrogen or an alkyl group having 1-6 carbons[;] and R¹⁶ and R¹⁷, which may be the same or different,] are independently alkyl groups having 1-6 carbons, or R¹⁶ and R¹⁷ taken in combination complete a five or six membered saturated ring;

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α is 0 or 1 and β is 0 or 1 provided that $\alpha + \text{all } \beta = 1$;

R^2 is selected from the group consisting of $-L-R_x$, $-L-S_c$, TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by [sulfonate] sulfo, carboxy, or amino; [or R^2 is $-L-R_x$ or $-L-S_c$; or TAIL; or BRIDGE-DYE]

$n = 0, 1$ or 2 ;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen [H];, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons[; or] a halogen[; or], a CYCLIC SUBSTITUENT[; or], $-OR^8$, $-SR^8$, $-(NR^8R^9)$ [; or], TAIL[; or], BRIDGE[-DYE; or], $-L-R_x$ [; or] and $-L-S_c$ [;] wherein R^8 and R^9 [, which can be the same or different,] are independently alkyl groups having 1-6 carbons[;] or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-V-(CH_2)_2-$ where V is $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring $[-R^{11}=R^{12}-R^{13}=R^{14}-]$ wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are [optionally and] independently selected from the group consisting of hydrogen, halogen, [alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or] $-OR^8$, $-SR^8$, [or] $-(NR^8R^9)$ [; or] a CYCLIC SUBSTITUENT[; or] a, TAIL[; or], BRIDGE[-DYE; or], $-L-R_x$ [; or], $-L-S_c$ [;] and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

R^5 is selected from the group consisting of [an alkyl that is saturated or unsaturated,

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linear or branched, having 1-6 carbons; or R^5 is a] CYCLIC SUBSTITUENT[; or R^5 is],
TAIL[; or], BRIDGE[-DYE; or], -L- R_x [; or], -L- S_c [; or R^5 is], a pair of electrons,
sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or
branched;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of hydrogen, [H,
C₁-C₆] alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, [or] and
heteroaryl; and

when present, BRIDGE is attached to a DYE compound provided that no more than one
of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond[,] or a covalent linkage [that is
linear or branched, cyclic or heterocyclic, saturated or unsaturated,] having [1-16] 1-20
nonhydrogen atoms selected from the group consisting of C, N, [P,] O and S[, such that
the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or
single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen,
phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or
heteroaromatic bonds];

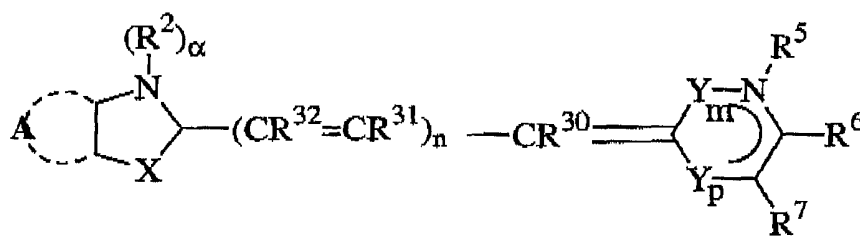
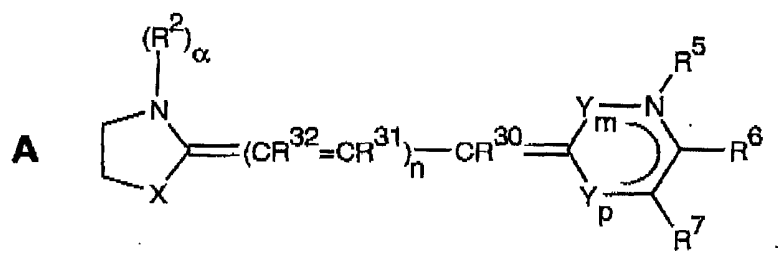
R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound [of the] having formula

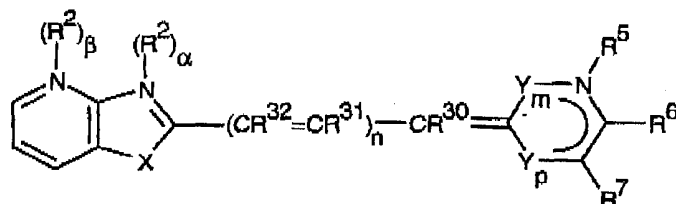
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wherein A, X, R^2 , α , n, Y_m , Y_p , $[R^3, R^4]$, R^5 , R^6 , R^7 , $[R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}]$, R^{30} , R^{31} , and R^{32} , [TAIL, CYCLIC SUBSTITUENT] are as defined above provided that BRIDGE not be any of $R^2, R^3, R^4, R^5, R^6, R^7, R^{11}, R^{12}, R^{13}, R^{14}$ and R^{15} [:].

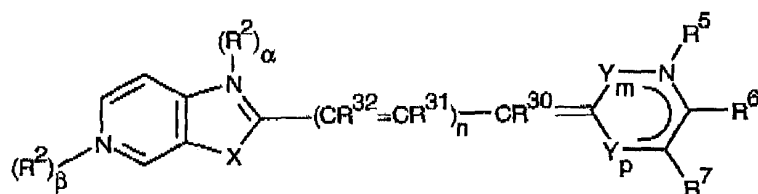
[that is bound to BRIDGE at one of R^3, R^4, R^5, R^6 , or R^7 .]

2. (Amended) [A] The compound[, as claimed in] according to Claim 1, having the formula selected from the group consisting of

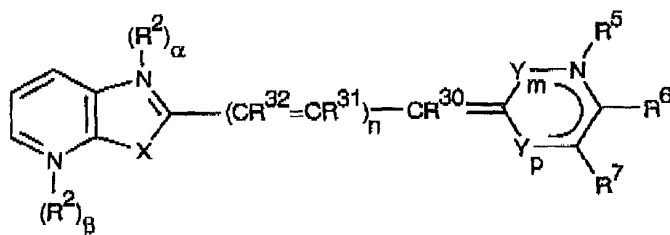


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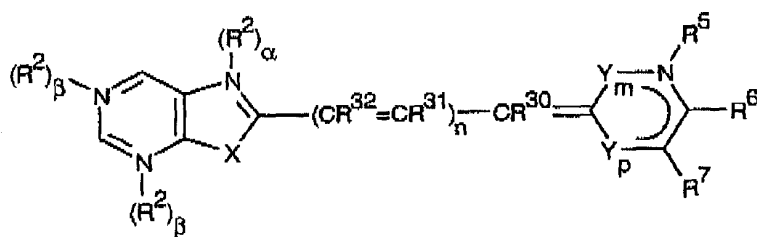
[the formula]



[the formula]

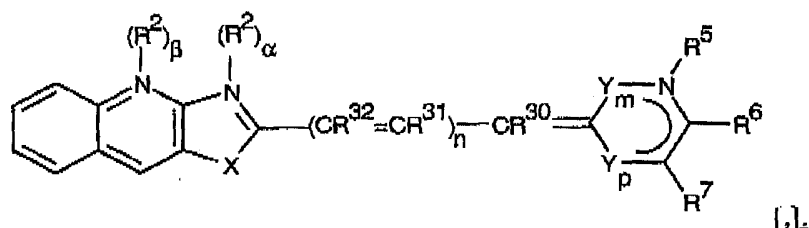


[the formula]



[or the formula] and

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[wherein either α or a β is 1.]

3. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein said TAIL [is a heteroatom-containing moiety having the] comprises formula LINK-SPACER-CAP;

wherein

LINK is a single covalent bond, -O-, -S-, or -NR²⁰-; where R²⁰ is [H] hydrogen, a linear or branched alkyl having 1-8 carbons, or [R²⁰ is] -SPACER'-CAP';

SPACER and SPACER'[, which may be the same or different] are individually covalent linkages[, that are linear or branched, cyclic or heterocyclic, saturated or unsaturated, [each] having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S[, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds];

CAP and CAP'[, which may be the same or different,] are individually -O-R²¹, -S-R²¹, -NR²¹R²², or -N⁺R²¹R²²R²³Ψ⁻;

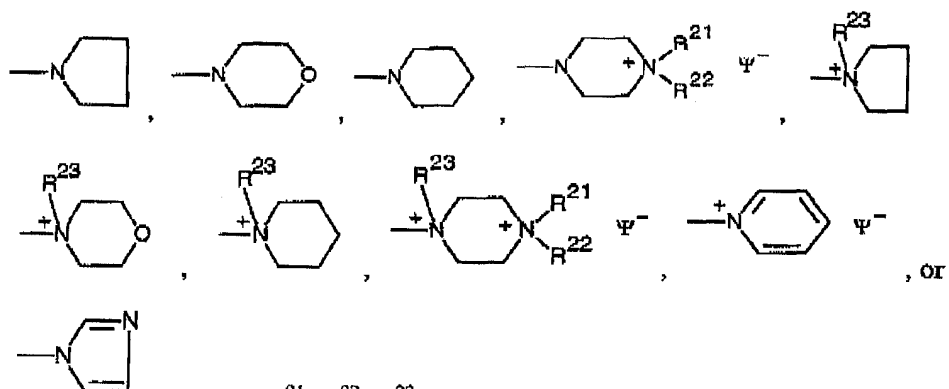
wherein

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R^{21} , R^{22} , and R^{23} are independently [H.] hydrogen or a linear or branched alkyl having 1-6 carbons or cycloalkyl having [1-8] 3-8 carbons, wherein said alkyl is optionally further substituted by substituents selected from the group consisting of halogen, hydroxy, alkoxy having 1-8 carbons, [carboxyalkyl having 1-8 carbons,], amino, carboxy, sulfo [or] and phenyl, wherein said phenyl is optionally further substituted by substituents selected from the group consisting of halogen, hydroxy, alkoxy having 1-8 carbons, aminoalkyl having 1-8 carbons, sulfoalkyl [or] and carboxyalkyl having 1-8 carbons; or[,] one or more of R^{21} , R^{22} and R^{23} , taken in combination with SPACER [or SPACER' or] and R^{20} or SPACER alone forms a 5- or 6-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring, the heteroatoms selected from O, N or S; where Ψ^- is a compatible counterion;

or

CAP and CAP' are independently



[; where R^{21} , R^{22} , R^{23} , and Ψ^- are as defined previously].

4. (Amended) [A] The compound[, as claimed in] according to Claim [1] 2, wherein each R^2 is independently ethyl or methyl, each X is independently O or S, each n is independently 0 or 1, and R^{30} , R^{31} , and R^{32} are each [H] hydrogen.

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5. (Amended) [A] The compound[, as claimed in] according to Claim [1] 2, wherein at least one R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , and R^{14} is a CYCLIC SUBSTITUENT [that is a substituted or unsubstituted] selected from the group consisting of [naphthyl, phenyl, thienyl,] aryl, heteroaryl, [or] and cycloalkyl having [3-8] 3-10 carbons wherein said CYCLIC SUBSTITUENT is individually and optionally substituted by TAIL, halogen, amino, or an alkyl containing moiety comprising 1-6 carbons.
6. (Amended) [A] The compound[, as claimed in] according to Claim [1] 3, wherein said TAIL comprises LINK that is a single covalent bond or NR^{20} wherein R^{20} is hydrogen or an alkyl; SPACER and SPACER' that are independently a linear alkyl having 1-8 carbons or a 6-membered carbon ring; CAP and CAP'[, which may be the same or different,] that are individually $-NR^{21}R^{22}$, or $-N^+R^{21}R^{22}R^{23} \Psi^-$, wherein R^{21} , R^{22} , and R^{23} are independently hydrogen, [H, or a linear or branched] alkyl or cycloalkyl [having 1-8 carbons; R^{20} is H or a linear or branched alkyl having 1-8 carbons; and SPACER and SPACER' are independently linear alkylenes alkyl having 1-8 carbons; or incorporate a phenylene ring].
7. (Amended) [A] The compound[, as claimed in] according to Claim [1] 6, wherein R^4 is a TAIL or BRIDGE[-DYE].
8. (Amended) [A] The compound[, as claimed in] according to Claim [1] 6, wherein R^5 is a TAIL[; or], a CYCLIC SUBSTITUENT[;], or BRIDGE[-DYE].
9. (Amended) [A] The compound[, as claimed in] according to Claim 8, wherein R^5 is a TAIL or a BRIDGE[-DYE, and] wherein TAIL and BRIDGE incorporate at least one quaternary nitrogen atom.
10. (Amended) [A] The compound[, as claimed in] according to Claim [1] 2, wherein each R^3 , R^{11} , R^{12} , R^{13} and R^{14} is hydrogen.

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11. (Amended) [A] The compound[, as claimed in] according to Claim [1] 3, wherein

R^5 is [a linear or branched] an alkyl [having 1-6 carbons;] and

R^4 is selected from the group consisting of halogen, [a] CYCLIC SUBSTITUENT, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, TAIL, BRIDGE[-DYE], $-L-R_x$, [or] and $-L-S_c$.

12. (Amended) [A] The compound[, as claimed in] according to Claim 1, wherein S_c is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, an oligonucleotide, a nucleic acid, a lipid, a polymeric microparticle, a biological cell, a DNA-binding protein [or] and a virus.

13. (Amended) [A] The compound[, as claimed in] according to Claim [1] 12, wherein S_c is an oligonucleotide, a nucleic acid, or a DNA-binding protein.

14. (Amended) [A] The compound[, as claimed in] according to Claim [2] 6, wherein

each X is O;

each n is independently = 0, 1, or 2;

each m = 1;

each R^{30} , R^{31} , and R^{32} are H; and

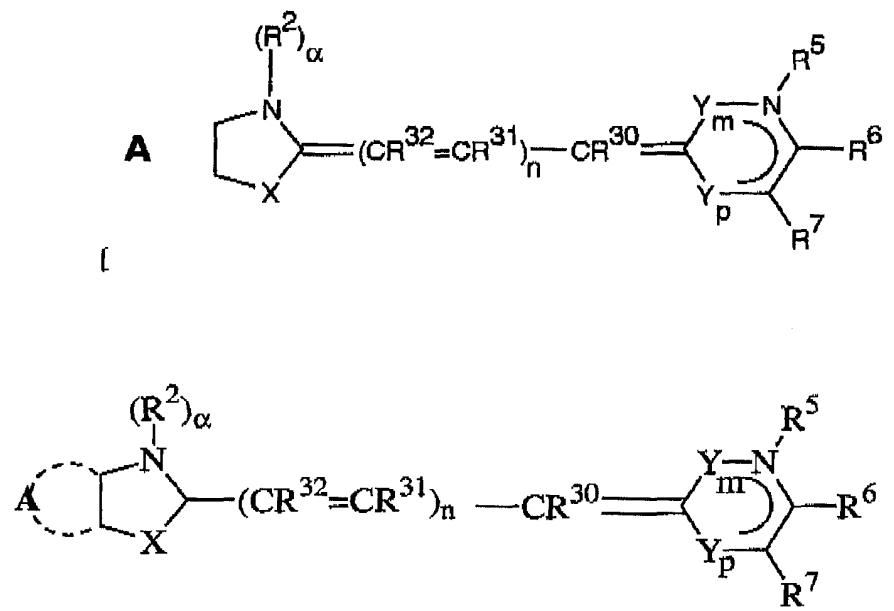
R^5 is selected from the group consisting of [a linear or branched] an alkyl [having 1-6 carbons], a TAIL, a CYCLIC SUBSTITUENT, [or] and a BRIDGE[-DYE].

15. (Amended) A [fluorescent] complex comprising: [a nucleic acid polymer non-

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covalently associated with one or more dye molecules, which may be the same or different, having the formula]

a) a compound having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R²)^β, provided at least one of [which] said ring atoms is [a nitrogen atom] N(R²)^β], said ring or rings being wherein aromatic carbons are optionally [further] substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, [or] -L- -R_x[; or] and -L-S_z;

X is selected from the group consisting of O, S, Se, NR¹⁵, [or] and CR¹⁶R¹⁷], wherein R¹⁵ is [H] hydrogen or an alkyl group having 1-6 carbons[;] and R¹⁶ and R¹⁷[, which may be the same or different,] are independently alkyl groups having 1-6 carbons, or R¹⁶ and R¹⁷ taken in combination complete a five or six membered saturated ring;

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α is 0 or 1 and β is 0 or 1 provided that $\alpha + \text{all } \beta = 1$;

R^2 is selected from the group consisting of $-L-R_x$, $-L-S_c$, TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by [sulfonate] sulfo, carboxy, or amino; [or R^2 is $-L-R_x$ or $-L-S_c$; or TAIL; or BRIDGE-DYE]

$n = 0, 1$ or 2 ;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen [H]; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; [or] a halogen; [or] a CYCLIC SUBSTITUENT; [or] $-OR^8$, $-SR^8$, $-(NR^8R^9)$; [or] TAIL; [or] BRIDGE[-DYE; or] $-L-R_x$; [or] and $-L-S_c$; [or] wherein R^8 and R^9 , which can be the same or different, are independently alkyl groups having 1-6 carbons; [or] 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-V-(CH_2)_2-$ where V is $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring $[-R^{11}=R^{12}-R^{13}=R^{14}-]$ wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are [optionally and] independently selected from the group consisting of hydrogen, halogen, [alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or] $-OR^8$, $-SR^8$, [or] $-(NR^8R^9)$; [or] a CYCLIC SUBSTITUENT; [or] a TAIL; [or] BRIDGE[-DYE; or] $-L-R_x$; [or] $-L-S_c$; and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

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R^5 is selected from the group consisting of [an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or R^5 is a] CYCLIC SUBSTITUENT[; or R^5 is], TAIL[; or], BRIDGE[-DYE; or], -L- R_x [; or], -L- S_c [; or R^5 is], a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of hydrogen [H, C₁-C₆] alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, [or] and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond[,] or a covalent linkage [that is linear or branched, cyclic or heterocyclic, saturated or unsaturated,] having [1-16] 1-20 nonhydrogen atoms selected from the group consisting of C, N, [P,] O and S[, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds];

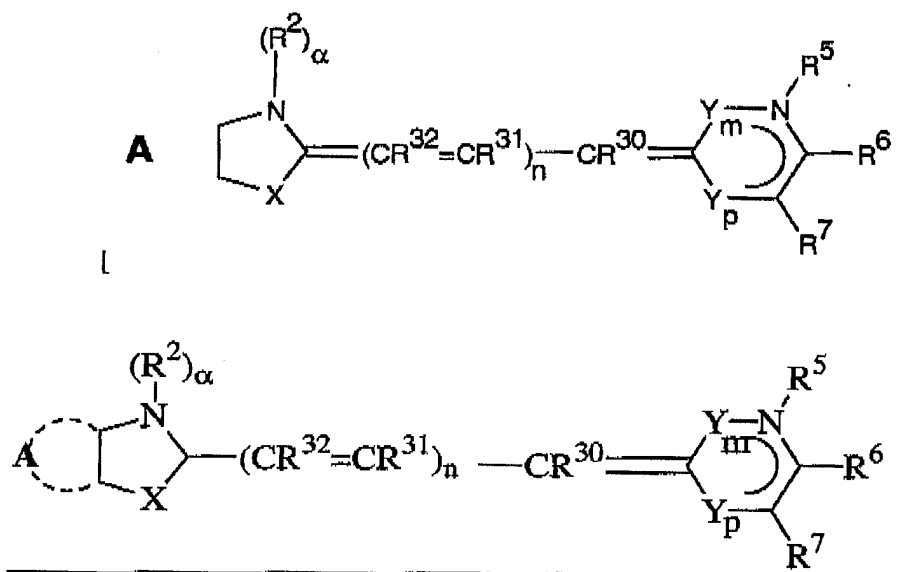
R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

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DYE is a compound [of the] having formula



wherein A, X, R², α, n, Y_m, Y_p, [R³, R⁴,] R⁵, R⁶, R⁷, [R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, R²¹, R²², R²³, R²⁴,] R³⁰, R³¹,] and R³², [TAIL, CYCLIC SUBSTITUENT] are as defined above provided that BRIDGE not be any of R², R³, R⁴, R⁵, R⁶, R⁷, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵ [;].

[that is bound to BRIDGE at one of R³, R⁴, R⁵, R⁶, or R⁷.] and

b) a nucleic acid polymer.

16. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim 15, wherein [the] said nucleic acid polymer is a chromosome or fragment thereof, or a natural or synthetic oligonucleotide.

17. (Cancelled) A fluorescent complex, as claimed in Claim 15, wherein said complex is

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present in an electrophoretic matrix or in a flowing medium.

18. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim [15] 16, wherein said nucleic acid is [obtained from a biological fluid] enclosed in a biological structure, free in solution, immobilized on a solid or semi-solid material or is extracted from a biological structure.

19. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim [15] 18, wherein said complex is enclosed in a biological structure[, or] present in [an aqueous or aqueous miscible] a solution or on an inert matrix.

20. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim 19 or 21, wherein said complex is enclosed in a biological structure [that is a cell].

21. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim [20] 18, wherein said biological structure is a cell and said cell is undergoing apoptosis, necrosis, or is in a cycle of [growth or] cell division.

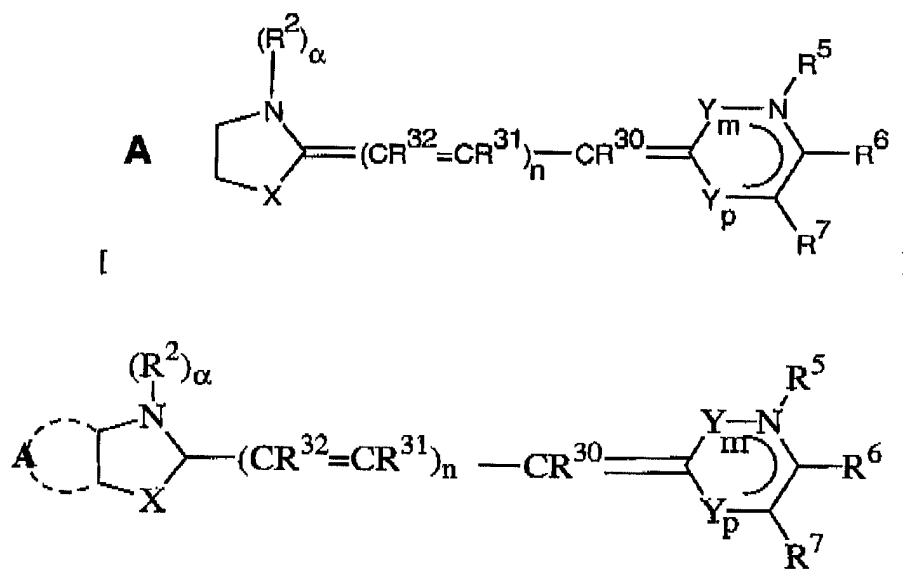
22. (Amended) [A fluorescent] The complex[, as claimed in] according to Claim 15, wherein at least one [dye molecule] compound is substituted by -L-Sc[,] wherein Sc is selected from the group consisting of [a] hapten, [a] nucleotide, [an] oligonucleotide, [a] nucleic acid polymer, [a] protein, [or a] polysaccharide and DNA binding protein.

23. (Cancelled) A compound, as claimed in Claim 22, wherein Sc is an oligonucleotide, a nucleic acid, or a DNA-binding protein.

24. (Amended) A [composition] complex comprising:

a) [one or more cyanine dyes] a compound having [the] formula;

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wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R²)^β, provided at least one of [which] said ring atoms is [a nitrogen atom] N(R²)^β], said ring or rings being] wherein aromatic carbons are optionally [further] substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, [or] -L-, -R_x[; or] and -L-S_c;

X is selected from the group consisting of O, S, Se, NR¹⁵, [or] and CR¹⁶R¹⁷], wherein R¹⁵ is H or an alkyl group having 1-6 carbons[;] and R¹⁶ and R¹⁷[, which may be the same or different,] are independently alkyl groups having 1-6 carbons, or R¹⁶ and R¹⁷ taken in combination complete a five or six membered saturated ring;

α is 0 or 1 and β is 0 or 1 provided that α + all β = 1;

R² is selected from the group consisting of -L-R_x-, -L-S_c-, TAIL, BRIDGE and an alkyl

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group having 1-6 carbons that is optionally substituted by [sulfonate] sulfo, carboxy, or amino; [or R^2 is $-L-R_x$ or $-L-S_c$; or TAIL; or BRIDGE-DYE]

$n = 0, 1$ or 2 ;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen [H]; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons[; or] a halogen[; or] a CYCLIC SUBSTITUENT[; or] $-OR^8$, $-SR^8$, $-(NR^8R^9)$ [; or] TAIL[; or] BRIDGE[-DYE; or] $-L-R_x$ [; or] and $-L-S_c$;] wherein R^8 and R^9 , which can be the same or different, are independently alkyl groups having 1-6 carbons[; or] 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-V-(CH_2)_2-$ where V $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring $[-R^{11}=R^{12}-R^{13}=R^{14}-]$ wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are [optionally and] independently selected from the group consisting of hydrogen, halogen, [alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or] $-OR^8$, $-SR^8$, [or] $-(NR^8R^9)$ [; or] a CYCLIC SUBSTITUENT[; or] a, TAIL[; or] BRIDGE[-DYE; or] $-L-R_x$ [; or] $-L-S_c$; and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

R^5 is selected from the group consisting of [an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or R^5 is a] CYCLIC SUBSTITUENT[; or R^5 is] a TAIL[; or] BRIDGE[-DYE; or] $-L-R_x$ [; or] $-L-S_c$]; or R^5 is] a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or

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branched;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of hydrogen, [H, C₁-C₆] alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, [or] and heteroaryl; and

when present, BRIDGE is attached to a DYE compound provided that no more than one of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond[,] or a covalent linkage [that is linear or branched, cyclic or heterocyclic, saturated or unsaturated,] having [1-16] 1-20 nonhydrogen atoms selected from the group consisting of C, N, [P,] O and S[, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds];

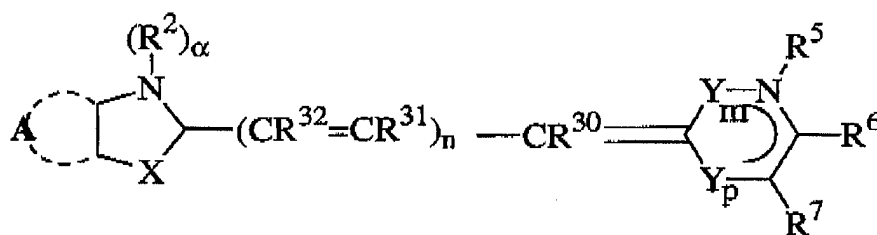
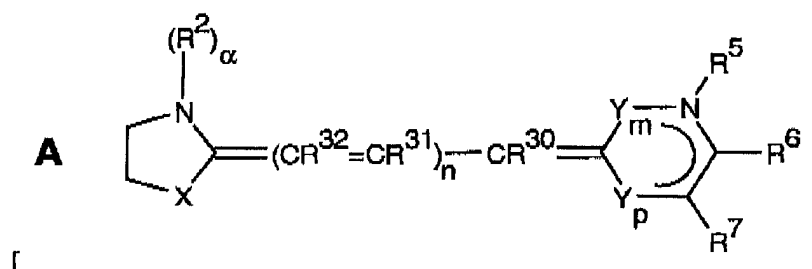
R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound [of the] having formula

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wherein A, X, R^2 , α , n, Y_m , Y_p , [R^3 , R^4], R^5 , R^6 , R^7 , [R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , R^{18} , R^{19} , R^{20} , R^{21} , R^{22} , R^{23} , R^{24}], R^{30} , R^{31} [,] and R^{32} [, TAIL, CYCLIC SUBSTITUENT] are as defined above provided that BRIDGE not be any of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} ; and,

[that is bound to BRIDGE at one of R^3 , R^4 , R^5 , R^6 , or R^7 .]

[b) a detergent; and]

[c)] b) a poly(amino acid)[,],

[in a cell-free aqueous solution where said detergent is present at a concentration less than the critical micelle concentration for that detergent.]

25. (Amended) [A] The [composition, as claimed in] complex according to Claim [24] 51, where said detergent is an alkyl sulfate or alkyl sulfonate salt.

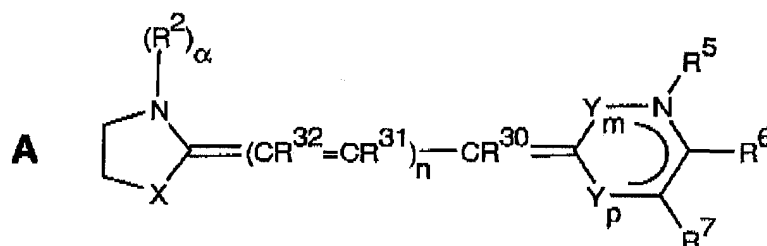
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26. (Amended) [A] The [composition, as claimed in] complex according to Claim [24] 25, wherein said poly(amino acids) are present on or in a solid or semi-solid matrix.

27. (Amended) [A] The [composition, as claimed in] complex according to Claim 26, wherein said matrix is a membrane or an electrophoretic gel.

28. (Withdrawn) A method of staining poly(amino acids), comprising the steps of:

a) combining a sample that contains or is thought to contain a poly(amino acid) with a staining mixture that contains one or more dyes having the formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring, at least one of which is a nitrogen atom, said ring or rings being optionally further substituted one or more times by alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, or -L-R_x; or -L-S_c;

X is O, S, Se, NR¹⁵, or CR¹⁶R¹⁷, where R¹⁵ is H or an alkyl group having 1-6 carbons; and R¹⁶ and R¹⁷, which may be the same or different, are independently alkyl groups having 1-6 carbons, or R¹⁶ and R¹⁷ taken in combination complete a five or six membered saturated ring;

α is 0 or 1;

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R^2 is an alkyl group having 1-6 carbons that is optionally substituted by sulfonate, carboxy, or amino; or R^2 is $-L-R_x$ or $-L-S_c$; or TAIL; or BRIDGE-DYE;

$n = 0, 1$ or 2 ;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently H; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or a halogen; or a CYCLIC SUBSTITUENT; or $-OR^8$, $-SR^8$, $-(NR^8R^9)$; or TAIL; or BRIDGE-DYE; or $-L-R_x$; or $-L-S_c$; where R^8 and R^9 , which can be the same or different, are independently alkyl groups having 1-6 carbons; or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-V-(CH_2)_2-$ where V is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 form a fused aromatic ring $-R^{11}=R^{12}-R^{13}=R^{14}-$ wherein R^{11} , R^{12} , R^{13} , and R^{14} are optionally and independently alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or $-OR^8$, $-SR^8$, or $-(NR^8R^9)$; or a CYCLIC SUBSTITUENT; or a TAIL; or BRIDGE-DYE; or $-L-R_x$; or $-L-S_c$;

R^5 is an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or R^5 is a CYCLIC SUBSTITUENT; or R^5 is TAIL; or BRIDGE-DYE; or $-L-R_x$; or $-L-S_c$; or R^5 is a pair of electrons;

R^{30} , R^{31} , and R^{32} are independently H, C_1 - C_6 alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, or heteroaryl;

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wherein

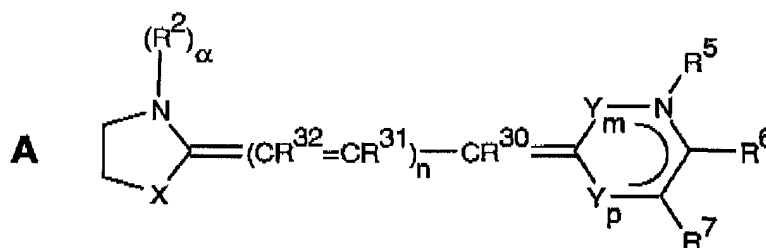
L and BRIDGE are independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds;

R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula



wherein A, X, R², α, n, Y_m, Y_p, R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷, R¹⁸, R¹⁹, R²⁰, R²¹, R²², R²³, R²⁴, R³⁰, R³¹, R³², TAIL, CYCLIC SUBSTITUENT are as defined above;

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that is bound to BRIDGE at one of R^3 , R^4 , R^5 , R^6 , or R^7 ;

b) incubating the combined mixture for a time sufficient for the dye in the staining mixture to associate with the poly(amino acid) in the sample mixture to form a dye-poly(amino acid) complex that gives a detectable optical response upon illumination;

d) illuminating said dye-poly(amino acid) complex; and

e) observing said detectable optical response.

29. (Withdrawn) A method, as claimed in Claim 28, further comprising heating the sample mixture prior to combining with the staining mixture, or heating the combined mixture.

30. (Withdrawn) A method, as claimed in Claim 28, further comprising removing, destroying, or dispersing below the critical micelle concentration any biological membranes that are present in the sample mixture.

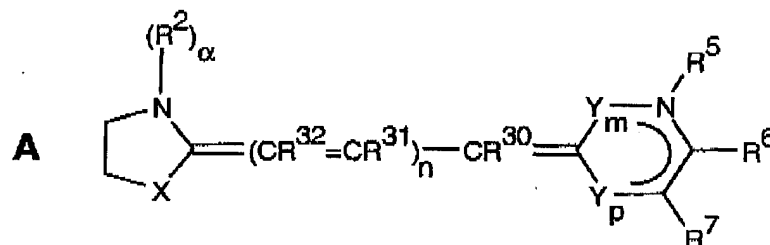
31. (Withdrawn) A method, as claimed in Claim 28, further comprising adding an anionic detergent to the sample mixture, staining mixture or combined mixture.

32. (Withdrawn) A method, as claimed in Claim 31, wherein said detergent is an alkyl sulfate or alkyl sulfonate salt having 6-18 carbons; that is present in a concentration of less than 0.1% by weight.

33. (Withdrawn) A method, as claimed in Claim 28, wherein said detectable optical response is a colorimetric response.

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34. (Withdrawn) A method, as claimed in Claim 28, wherein said detectable optical response is a fluorescence response.
35. (Withdrawn) A method, as claimed in Claim 28, further comprising quantitating said poly(amino acid) by measuring said detectable optical response and comparing said measurement with a standard.
36. (Withdrawn) A method, as claimed in Claim 28, further comprising electrophoretically separating the sample mixture before, after, or while it is combined with the staining mixture.
37. (Withdrawn) A method, as claimed in Claim 28, further comprising transferring the sample mixture to a solid or semi-solid matrix before or after combining with the staining mixture.
38. (Withdrawn) A method, as claimed in Claim 28, further comprising adding an additional reagent to the sample mixture, the staining mixture, or the combined mixture.
39. (Withdrawn) A method of staining nucleic acids, comprising
- a) combining a sample that contains or is thought to contain a nucleic acid with a mixture containing a dye compound of the formula



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wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring, at least one of which is a nitrogen atom, said ring or rings being optionally further substituted one or more times by alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, or -L-R_x; or -L-S_c;

X is O, S, Se, NR¹⁵, or CR¹⁶R¹⁷, where R¹⁵ is H or an alkyl group having 1-6 carbons; and R¹⁶ and R¹⁷, which may be the same or different, are independently alkyl groups having 1-6 carbons, or R¹⁶ and R¹⁷ taken in combination complete a five or six membered saturated ring;

α is 0 or 1;

R² is an alkyl group having 1-6 carbons that is optionally substituted by sulfonate, carboxy, or amino; or R² is -L-R_x or -L-S_c; or TAIL; or BRIDGE-DYE;

n = 0, 1 or 2;

Y is -CR³=CR⁴-;

p and m = 0 or 1, such that p + m = 1;

R³, R⁴, R⁶, and R⁷ are independently H; an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or a halogen; or a CYCLIC SUBSTITUENT; or -OR⁸, -SR⁸, -(NR⁸R⁹); or TAIL; or BRIDGE-DYE; or -L-R_x; or -L-S_c; where R⁸ and R⁹, which can be the same or different, are independently alkyl groups having 1-6 carbons; or 1-2 alicyclic or aromatic rings; or R⁸ and R⁹ taken in combination are -(CH₂)₂-V-(CH₂)₂- where V is a single bond, -O-, -CH₂-, or -NR¹⁰-, where R¹⁰ is H or an alkyl having 1-6 carbons;

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or R^6 and R^7 form a fused aromatic ring $-R^{11}=R^{12}-R^{13}=R^{14}-$ wherein R^{11} , R^{12} , R^{13} , and R^{14} are optionally and independently alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or $-OR^8$, $-SR^8$, or $-(NR^8R^9)$; or a CYCLIC SUBSTITUENT; or a TAIL; or BRIDGE-DYE; or $-L-R_x$; or $-L-S_c$;

R^5 is an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or R^5 is a CYCLIC SUBSTITUENT; or R^5 is TAIL; or BRIDGE-DYE; or $-L-R_x$; or $-L-S_c$; or R^5 is a pair of electrons;

R^{30} , R^{31} , and R^{32} are independently H, C_1 - C_6 alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, or heteroaryl;

wherein

L and BRIDGE are independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-16 nonhydrogen atoms selected from the group consisting of C, N, P, O and S, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds;

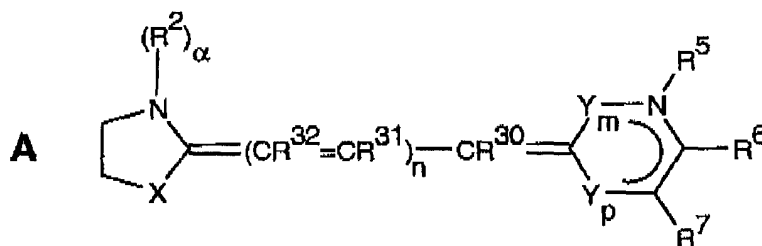
R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula

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wherein A, X, R^2 , α , n, Y_m , Y_p , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , R^{18} , R^{19} , R^{20} , R^{21} , R^{22} , R^{23} , R^{24} , R^{30} , R^{31} , R^{32} , TAIL, CYCLIC SUBSTITUENT are as defined above;

that is bound to BRIDGE at one of R^3 , R^4 , R^5 , R^6 , or R^7 ;

b) incubating the sample and mixture for a time sufficient for the dye compound to combine with the nucleic acid in the sample to form one or more dye-nucleic acid complexes that give a detectable fluorescent signal.

40. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein said sample or said mixture comprises an electrophoretic gel.

41. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein the sample comprises a biological fluid.

42. (Withdrawn) A method of staining nucleic acids, as claimed in Claim 39, wherein the sample comprises cells.

43. (Withdrawn) A method of staining nucleic acids, according to Claim 39, where the sample comprises cell-free nucleic acids.

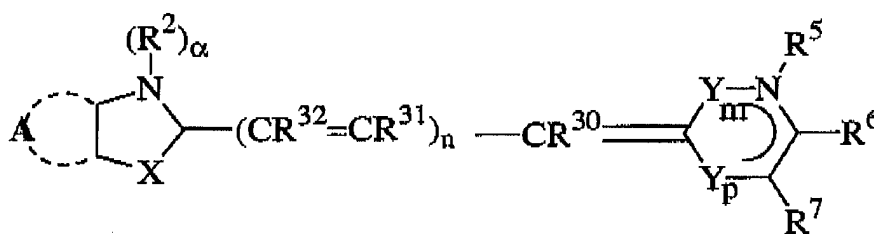
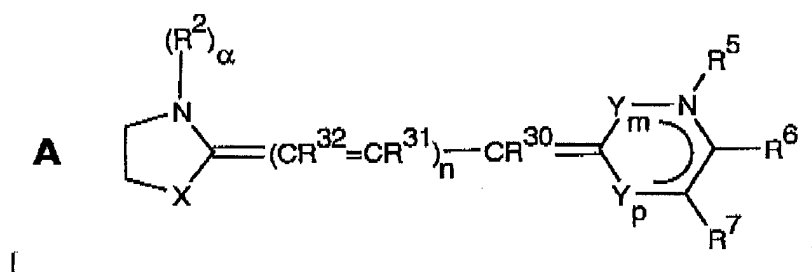
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44. (Amended) A kit[,] comprising:

a) a stock solution comprising:

i) one or more compounds individually having formula:

[a) a compound of the formula]



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R²)^β, provided at least one of [which] said ring atoms is [a nitrogen atom] N(R²)^β[, said ring or rings being] wherein aromatic carbons are optionally [further] substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, [or] -L- -R_x[; or] and -L-S_c;

X is selected from the group consisting of O, S, Se, NR¹⁵, [or] and CR¹⁶R¹⁷[,] wherein R¹⁵ is [H] hydrogen or an alkyl group having 1-6 carbons[;] and R¹⁶ and R¹⁷, which may

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be the same or different,] are independently alkyl groups having 1-6 carbons, or R^{16} and R^{17} taken in combination complete a five or six membered saturated ring;

α is 0 or 1 and β is 0 or 1 provided that $\alpha + \text{all } \beta = 1$;

R^2 is selected from the group consisting of $-L-R_x$, $-L-S_c$, TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by [sulfonate] sulfo, carboxy, or amino; [or R^2 is $-L-R_x$ or $-L-S_c$; or TAIL; or BRIDGE-DYE]

$n = 0, 1$ or 2 ;

Y is $-\text{CR}^3=\text{CR}^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen [H];, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons[; or], a halogen[; or], a CYCLIC SUBSTITUENT[; or], $-\text{OR}^8$, $-\text{SR}^8$, $-(\text{NR}^8\text{R}^9)$ [; or], TAIL[; or], BRIDGE[-DYE; or], $-L-R_x$ [; or] and $-L-S_c$ [;] wherein R^8 and R^9 [, which can be the same or different,] are independently alkyl groups having 1-6 carbons[;] or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $[-(\text{CH}_2)_2-\text{V}-(\text{CH}_2)_2-$ where V] $-(\text{CH}_2)_2-\text{W}-(\text{CH}_2)_2-$ where W is a single bond, $-\text{O}-$, $-\text{CH}_2-$, or $-\text{NR}^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring $[-\text{R}^{11}=\text{R}^{12}-\text{R}^{13}=\text{R}^{14}-]$ wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are [optionally and] independently selected from the group consisting of hydrogen, halogen, [alkyl that are saturated or unsaturated, linear or branched, having 1-6 carbons; or] $-\text{OR}^8$, $-\text{SR}^8$, [or] $-(\text{NR}^8\text{R}^9)$ [; or] a CYCLIC SUBSTITUENT[; or] a, TAIL[; or], BRIDGE[-DYE; or], $-L-R_x$ [; or], $-L-S_c$ [;] and a saturated or unsaturated alkyl having 1-6 carbons that is linear or

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branched;

R^5 is selected from the group consisting of [an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons; or R^5 is a] CYCLIC SUBSTITUENT[; or R^5 is], TAIL[; or], BRIDGE[-DYE; or], -L- R_x [; or], -L- S_c [; or R^5 is], a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of H, [C₁-C₆] alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, [or] and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is BRIDGE;

wherein

L and BRIDGE are independently a single covalent bond[, or a covalent linkage [that is linear or branched, cyclic or heterocyclic, saturated or unsaturated,] having [1-16] 1-20 nonhydrogen atoms selected from the group consisting of C, N, [P,] O and S[, such that the linkage contains any combination of ether, thioether, amine, ester, amide bonds; or single, double, triple or aromatic carbon-carbon bonds; or phosphorus-oxygen, phosphorus-sulfur bonds, nitrogen-nitrogen or nitrogen-oxygen bonds; or aromatic or heteroaromatic bonds];

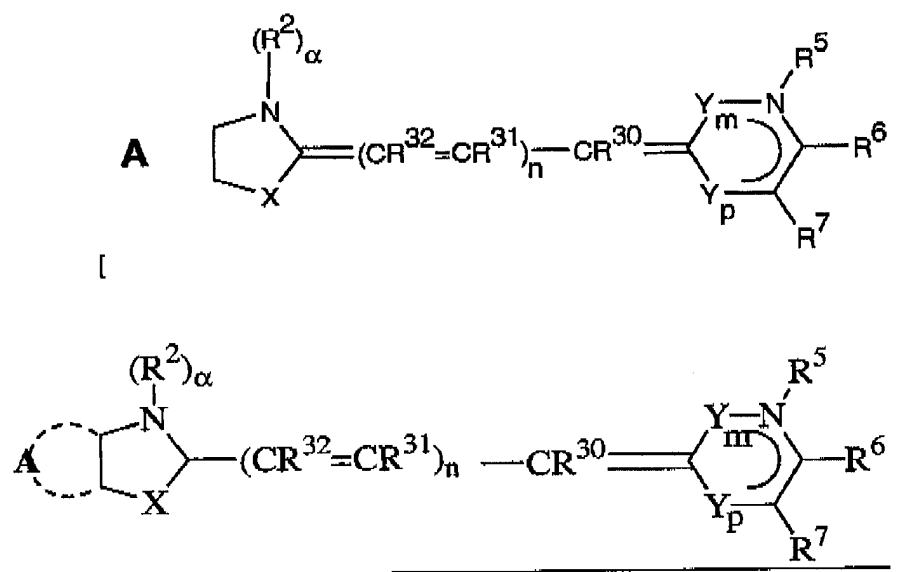
R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

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DYE is a compound [of the] having formula



wherein A, X, R^2 , α , n, Y^m , Y^p , $[R^3, R^4]$, R^5 , R^6 , R^7 , $[R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}]$, R^{30} , R^{31} , and R^{32} , TAIL, CYCLIC SUBSTITUENT] are as defined above provided that BRIDGE not be any of $R^2, R^3, R^4, R^5, R^6, R^7, R^{11}, R^{12}, R^{13}, R^{14}$ and R^{15} ;

[that is bound to BRIDGE at one of R^3, R^4, R^5, R^6 , or R^7 .]

[wherein said compound is present as a stock solution.]

ii) an organic solvent; and,

b) a buffer suitable for dilution of said stock solution.

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45. (Cancelled) A kit, as claimed in Claim 44, further comprising a buffer suitable for dilution of the stock solution.

46. (Amended) [A] The kit[, as claimed in] according to Claim 44, further comprising a [fluorescence] standard, [a nucleic acid, a poly(amino acid),] an additional detection reagent, a silicon chip, a glass slide, or any combination thereof.

47. (Amended) [A] The kit[, as claimed in] according to Claim [44] 46, wherein [the] said additional detection reagent is selected from the group consisting of an organelle stain, [an] a labeled immunoreagent, a drug, and an enzyme[, or an enzyme substrate].

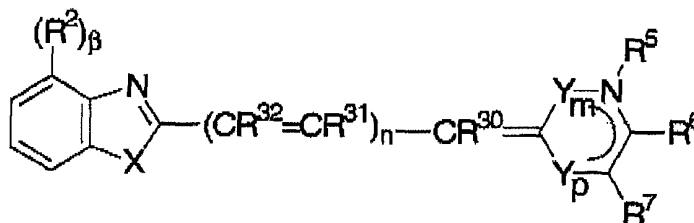
48. (New) The kit according to Claim 47, wherein said R^5 is BRIDGE that is attached to said DYE.

49. (New) The kit according to Claim 47, wherein said kit comprises two to six individual said compounds.

50. (New) The kit according to any one of claims Claim 47, 48 or 49, wherein said organic solvent is DMSO.

51. (New) The complex according to Claim 24, wherein said complex further comprises a detergent.

52. (New) A compound having formula:



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wherein R^2 is an alkyl having 1-6 carbon atoms and wherein meta and ortho positions to R^2 are independently and optionally substituted with halogen or an alkyl having 1-6 carbons atoms;

X is selected from the group consisting of O, S, Se, NR^{15} , and $CR^{16}R^{17}$ wherein R^{15} is H or an alkyl group having 1-6 carbons and R^{16} and R^{17} are independently alkyl groups having 1-6 carbons;

$n = 0, 1$ or 2 ;

$\beta = 1$;

Y is $-CR^3=CR^4-$;

p and m = 0 or 1, such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen, an alkyl having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, TAIL and BRIDGE wherein R^8 and R^9 are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are independently selected from the group consisting of hydrogen, halogen, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, CYCLIC SUBSTITUENT, TAIL, BRIDGE, and an alkyl having 1-6 carbons;

R^5 is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, sulfoalkyl and an alkyl having 1-6 carbons;

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R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of H, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

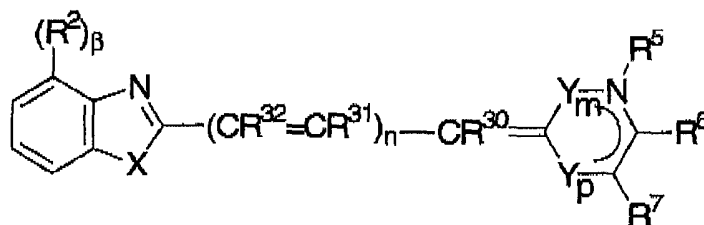
when present, BRIDGE is attached to a DYE compound provided that no more than one of R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} and R^{14} is BRIDGE;

wherein;

BRIDGE is independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

TAIL is a heteroatom-containing moiety;

DYE is a compound of the formula



wherein X, R^2 , n, Y_m , Y_p , R^5 , R^6 , R^7 , R^{30} , R^{31} and R^{32} are as defined above provided that BRIDGE not be any of R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} and R^{14} .

53. (New) The compound according to Claim 52, wherein

R^2 is methyl; said meta and ortho positions are optionally substituted by halogen; X is S or O; n = 0; m = 1;

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R^3 and R^4 are independently selected from the group consisting of H, alkyl, CYCLIC SUBSTITUENT and TAIL;

R^6 and R^7 taken in combination form a fused 6-membered aromatic ring wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are independently hydrogen, $-OR^8$, or an alkyl having 1-6 carbons wherein R^8 is methyl;

R^5 is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, and methyl;

R^{30} is hydrogen.

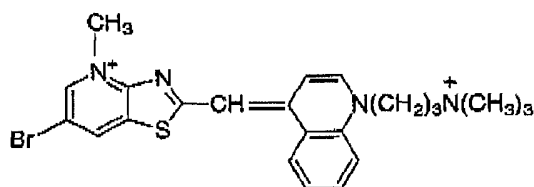
54. (New) The compound according to Claim 53, wherein X is S, said halogen is chlorine or bromine, R^3 is hydrogen, R^4 is hydrogen or an alkyl and R^5 is selected from the group consisting of methyl, CYCLIC SUBSTITUENT, BRIDGE and TAIL wherein said CYCLIC SUBSTITUENT is an unsubstituted aryl and TAIL comprises formula - $(CH_2)_3N(CH_3)_3$.

55. (New) The compound according to Claim 54 or 53, wherein said R^5 is BRIDGE comprising formula $-(CH_2)_3N(CH_3)CH_2-$.

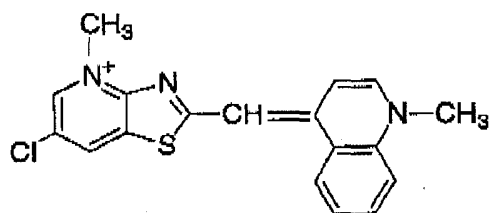
56. (New) The compound according to any one of Claims 55, 54 or 53, wherein said BRIDGE is attached to said DYE.

57. (New) The compound according to Claim 54, wherein said compound has the formula:

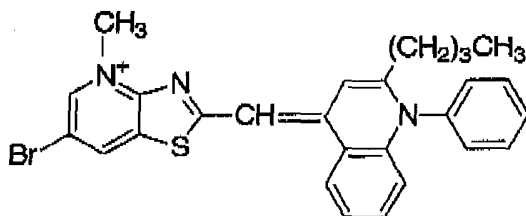
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58. (New) The compound according to Claim 54, wherein said compound has the formula:



59. (New) The compound according to Claim 54, wherein said compound has the formula:

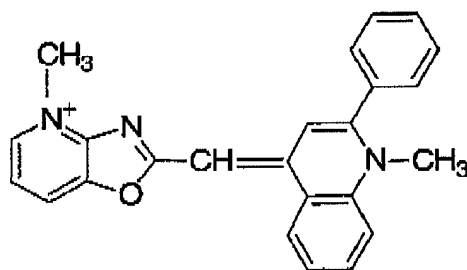


60. (New) The compound according to Claim 53, wherein X is O; R³ is hydrogen; R⁴ is selected from the group consisting of hydrogen, CYCLIC SUBSTITUENT, TAIL and an alkyl; R⁵ is selected from the group consisting of methyl, CYCLIC SUBSTITUENT, BRIDGE and TAIL wherein said CYCLIC SUBSTITUENT is an unsubstituted aryl and said TAIL comprises LINK that is a single covalent bond, SPACER that is a phenyl ring

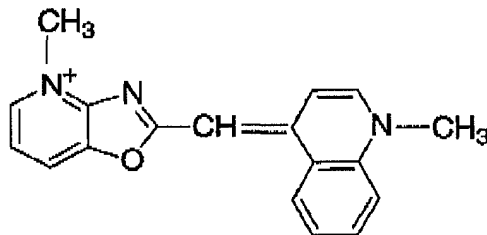
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and CAP comprising formula $-(CH_2)_2N^+CH_3(CH_2CH_3)_2$; R^{11} , R^{12} , R^{13} , and R^{14} are individually hydrogen or $-OCH_3$.

61. (New) The compound according to Claim 60, wherein said compound has the formula:

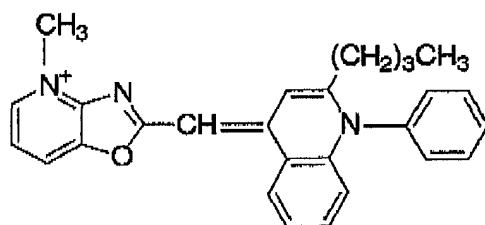


62. (New) The compound according to Claim 60, wherein said compound has the formula:

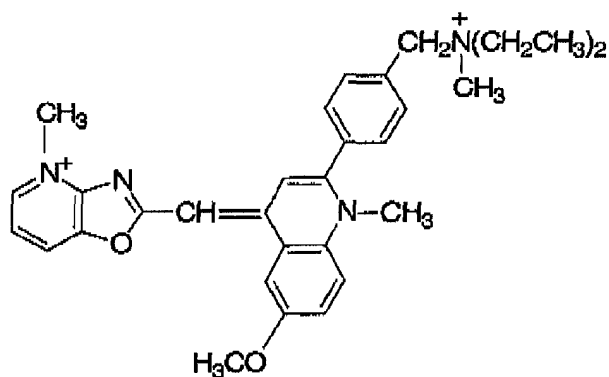


63. (New) The compound according to Claim 60, wherein said compound has the formula:

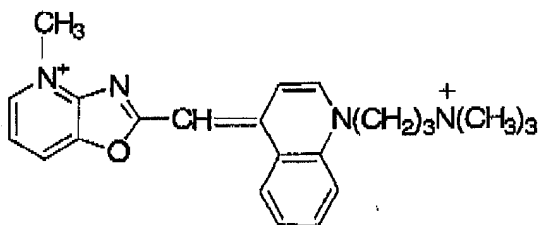
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64. (New) The compound according to Claim 60, wherein said compound has the formula:

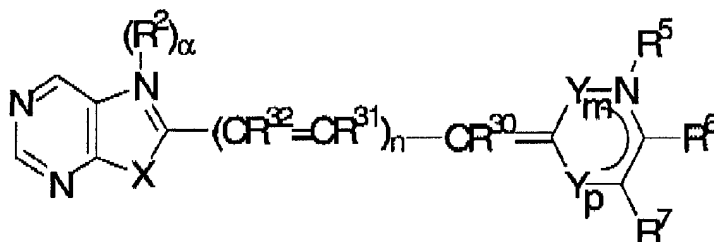


65. (New) The compound according to Claim 60, wherein said compound has the formula:



66. (New) A compound having formula:

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wherein R^2 is an alkyl having 1-6 carbon atoms and fused 6-membered aromatic ring is optionally substituted at a ring carbon by methylthio;

X is selected from the group consisting of O, S, Se, NR^{15} , and $CR^{16}R^{17}$ wherein R^{15} is hydrogen or an alkyl group having 1-6 carbons and R^{16} and R^{17} are independently alkyl groups having 1-6 carbons;

$n = 0, 1$ or 2 ;

$\alpha = 1$;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen, an alkyl having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, TAIL and BRIDGE wherein R^8 and R^9 are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are independently selected from the group consisting of hydrogen, halogen, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, CYCLIC SUBSTITUENT, TAIL, BRIDGE,

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and an alkyl having 1-6 carbons;

R^5 is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, sulfoalkyl and an alkyl having 1-6 carbons;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of H, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

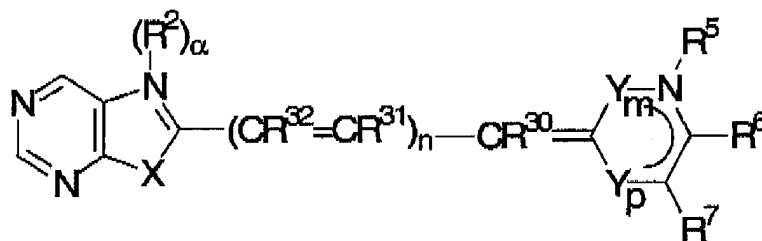
when present, BRIDGE is attached to a DYE compound provided that no more than one of R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} and R^{14} is BRIDGE;

wherein;

BRIDGE is independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

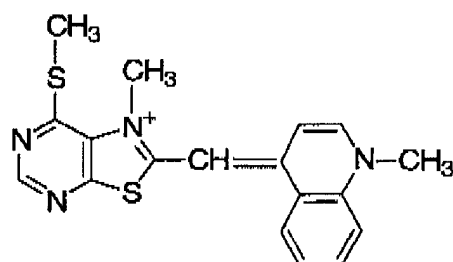


wherein X, R^2 , n, Y_m , Y_p , R^5 , R^6 , R^7 , R^{30} , R^{31} and R^{32} are as defined above provided that BRIDGE not be any of R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} and R^{14} .

67. (New) The compound according to Claim 66, wherein said 6-membered aromatic ring is substituted by methylthio; X is S; n is 0; m is 1; R^3 , R^4 , R^{11} , R^{12} , R^{13} and R^{14} are hydrogen and R^5 is methyl.

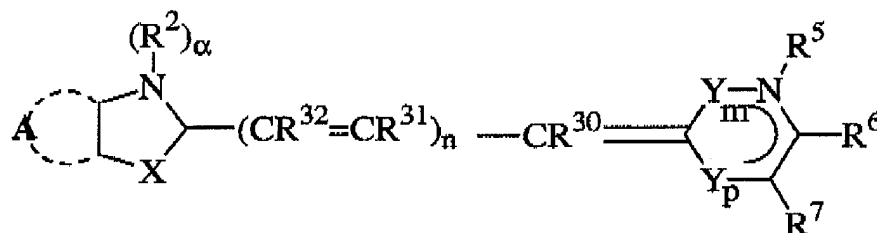
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68. (New) The compound according to Claim 67, wherein said compound has the formula:



69. (New) A solution for staining nucleic acid polymers or poly(amino acids) wherein said solution comprises:

a) one or more compounds having formula



wherein A represents the atoms necessary to form one to two fused aromatic rings having 6 atoms in each ring selected from the group consisting of -C-, CH or N(R²)^β, provided at least one of said ring atoms is N(R²)^β wherein aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, alkoxy having from 1-6 carbons, trifluoromethyl, halogen, methylthio, -L-R_x and -L-S_c;

X is selected from the group consisting of O, S, Se, NR¹⁵, and CR¹⁶R¹⁷ wherein R¹⁵ is

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hydrogen or an alkyl group having 1-6 carbons and R^{16} and R^{17} are independently alkyl groups having 1-6 carbons, or R^{16} and R^{17} taken in combination complete a five or six membered saturated ring;

α is 0 or 1 and β is 0 or 1 provided that $\alpha + \text{all } \beta = 1$;

R^2 is selected from the group consisting of $-L-R_x$, $-L-S_c$, TAIL, BRIDGE and an alkyl group having 1-6 carbons that is optionally substituted by sulfo, carboxy, or amino;

$n = 0, 1$ or 2 ;

Y is $-CR^3=CR^4-$;

p and $m = 0$ or 1 , such that $p + m = 1$;

R^3 , R^4 , R^6 , and R^7 are independently selected from the group consisting of hydrogen, an alkyl that is saturated or unsaturated, linear or branched, having 1-6 carbons, a halogen, a CYCLIC SUBSTITUENT, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, TAIL, BRIDGE, $-L-R_x$ and $-L-S_c$ wherein R^8 and R^9 are independently alkyl groups having 1-6 carbons or 1-2 alicyclic or aromatic rings; or R^8 and R^9 taken in combination are $-(CH_2)_2-W-(CH_2)_2-$ where W is a single bond, $-O-$, $-CH_2-$, or $-NR^{10}-$, where R^{10} is H or an alkyl having 1-6 carbons;

or R^6 and R^7 taken in combination form a fused 6-membered aromatic ring wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are independently selected from the group consisting of hydrogen, halogen, $-OR^8$, $-SR^8$, $-(NR^8R^9)$, a CYCLIC SUBSTITUENT, TAIL, BRIDGE, $-L-R_x$, $-L-S_c$ and a saturated or unsaturated alkyl having 1-6 carbons that is linear or branched;

R^5 is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, $-L-R_x$, $-L-S_c$, a pair of electrons, sulfoalkyl and a saturated or unsaturated alkyl having 1-

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6 carbons that is linear or branched;

R^{30} , R^{31} , and R^{32} are independently selected from the group consisting of hydrogen, alkyl having 1-6 carbons, cycloalkyl having 3-10 carbons, aryl, and heteroaryl; and,

when present, BRIDGE is attached to a DYE compound provided that no more than one of R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^{11} , R^{12} , R^{13} , R^{14} and R^{15} is BRIDGE;

wherein

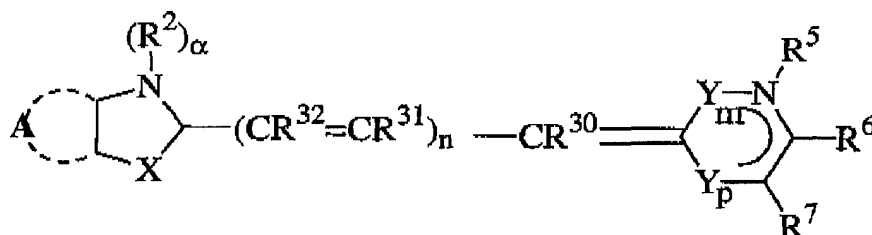
L and BRIDGE are independently a single covalent bond or a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O and S;

R_x is a reactive group;

S_c is a conjugated substance;

TAIL is a heteroatom-containing moiety;

DYE is a compound having formula

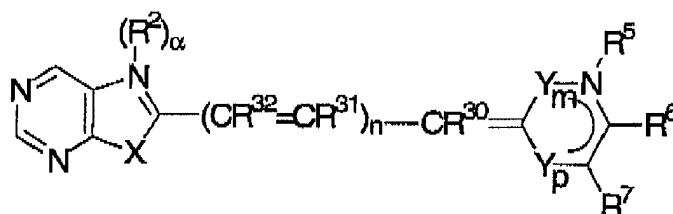


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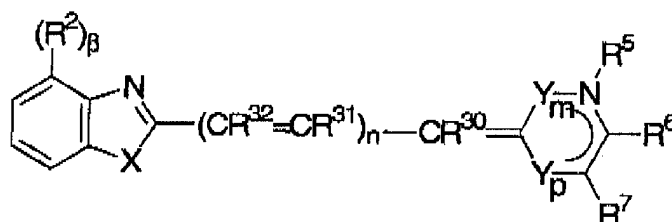
wherein A, X, R², α , n, Y_m, Y_p, R⁵, R⁶, R⁷, R³⁰, R³¹ and R³² are as defined above provided that BRIDGE not be any of R², R³, R⁴, R⁵, R⁶, R⁷, R¹¹, R¹², R¹³, R¹⁴ and R¹⁵; and,

b) an organic solvent.

70. (New) The solution according to Claim 69, wherein said compound has the formula



or



71. (New) The solution according to Claim 70, wherein R² is methyl; said 6-membered aromatic carbons are optionally substituted one or more times by substituents selected from the group consisting of hydrogen, alkyl having from 1-6 carbons, halogen, methylthio; X is S or O; n = 0; m = 1;

R³ and R⁴ are independently selected from the group consisting of hydrogen, alkyl, CYCLIC SUBSTITUENT and TAIL;

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R^6 and R^7 taken in combination form a fused 6-membered aromatic ring wherein ring substituents R^{11} , R^{12} , R^{13} , and R^{14} are independently hydrogen, $-OR^8$, or an alkyl having 1-6 carbons wherein R^8 is methyl;

R^5 is selected from the group consisting of CYCLIC SUBSTITUENT, TAIL, BRIDGE, a pair of electrons, and methyl;

R^{30} is hydrogen.

72. (New) The solution according to Claim 71, wherein R^3 is hydrogen, said CYCLIC SUBSTITUENT is an unsubstituted aryl and TAIL comprises LINK that is a single covalent bond, SPACER that is a phenyl ring or a linear alkyl and CAP comprising formula $-(CH)_2N^+CH_3(CH_2CH_3)_2$ or formula $-N(CH_3)_3$.

73. (New) The solution according to Claim 72, wherein R^5 is BRIDGE comprising formula $-(CH_2)_3N(CH_3)CH_2-$ wherein DYE is attached to said BRIDGE.

74. (New) The solution according to Claim 72 or 73 wherein said organic solvent is DMSO.

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Respectfully submitted,

Date: October 16, 2002

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